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Studies on the Limiting Amino Acids in Laying Hen Diets

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STUDIES ON THE LIMITING AMINO ACIDS IN LAYING HEN DIETS

BY

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STUDIES ON THE LIMITING AMINO ACIDS IN LAYING HEN DIETS
Abstract

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Under the supervision of Professor C. W. Carlson

Experiments were conducted over a period of two years to study the effects of amino acid supplementation of low and high energy and low and high protein diets on the performance of SCWL laying hens. This research was conducted in two different environmental conditions; one a litter floor, cold-wall poultry house, the other a warm-wall poultry house with individual cages.

In the litter floor, cold-wall environment, methionine and lysine were added singly and in combination to a 16 percent protein diet containing 1414 Calories of metabolizable energy per pound and methionine singly and a combination of methionine and lysine to a 16 percent protein diet containing 1340 Calories of metabolizable energy per pound. These were corn-soybean oil meal type diets.

The dietary treatments did not beneficially affect egg production, feed efficiency, body weight, egg weight, Haugh Units, fertility or hatchability of fertile eggs. In one experiment the addition of 0.1 percent L-lysine did improve the Haugh Units significantly over the basal control group.

With an 11 percent corn-soybean oil meal type ration containing 0.2 percent added DL-methionine the addition of 0.3 percent L-lysine slightly improved, though not significantly, egg production, feed

efficiency and egg weight. Haugh Units, fertility and hatchability of fertile eggs were not affected.

Egg production and feed efficiency for the methionine plus lysine supplemented 11 percent protein diet were inferior to that obtained with the 16 percent protein diets. In addition, the low protein groups lost body weight during the experimental periods while the high protein groups gained weight. Fertility and hatchability of fertile eggs were not affected by the protein levels.

In the warm-wall, individual cage environment, the effect of amino acid supplementation of low protein diets upon laying hen performance was studied. No benefit from the addition of methionine singly to a low protein, high energy diet (mostly corn) was observed. However, egg production and feed efficiency were improved with combined additions of 0.2 percent DL-methionine and 0.3 percent L-lysine to this diet. Body weight, egg weight and Haugh Units were not affected by the amino acid additions. Egg production and feed efficiency of the methionine plus lysine supplemented 11 percent diet were inferior to that obtained from hens receiving a 16 percent protein diet containing approximately the same level of energy. This indicated that other amino acids might also be limiting in the low protein diet.

Cumulative additions of methionine, lysine, glycine, valine, arginine, isoleucine and tryptophan were made to a 10 percent protein, low energy diet containing 1001 Calories of metabolizable energy per pound. Similarly, in another experiment, methionine, lysine, tryptophan, isoleucine, arginine and valine were cumulatively added to an 11 percent protein diet containing 1004 Calories of metabolizable energy per pound. In both of these experiments, methionine improved egg production and feed efficiency. None of the other amino acids affected the laying hen performance. The low protein groups lost body weight in both of the experiments while hens receiving a 16 percent protein diet gained weight. The low energy, low protein diets used in these experiments had a calorie-protein ratio and an amino acid make-up similar to that of the 16 percent protein diet discussed earlier. Since the addition of methionine to the low protein, low energy diet resulted in a response by improving egg production a similar response might be expected from the addition of methionine to the 16 percent protein diet. However, the research results discussed earlier did not indicate such a response. Perhaps the hens overconsumed on energy in order to meet their amino acid requirements.

When amino acids were added to an 11 percent protein, high energy diet containing 0.2 percent and 0.3 percent added DL-methionine and L-lysine respectively, responses of improved egg production, feed efficiency and body weight maintenance were obtained from the addition of 0.05 percent DL-tryptophan. Egg weight and Haugh Units were not significantly affected. This indicates that, in addition to methionine and lysine, tryptophan was also limiting in the high energy, low protein diet.

Studies on the Limiting Amino

Acids in Laying Hen Diets

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Doctor of Philosophy, and is acceptable as meeting the thesis requirements for this degree, but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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INTRODUCTION

During the last decade the laying hen's requirement for protein has received as much or more attention from poultry researchers than any other single nutrient. Considering the fact that the cost of protein makes up a large portion of the total feed cost of egg production, this attention is certainly justified. However, no field of poultry nutrition research appears to be yielding such contrasting data as have the studies on protein requirements of the laying hen. Requirements have been suggested from 11 to 18 percent of the diet with the National Research Council (1960) recommending a level of 15 percent of the ration.

The laying hen does not have a requirement for protein *per se* but rather for amino acids. As a result, the ability of the protein in the diet to provide the essential amino acids at a normal rate of feed intake becomes the determining factor. This may be influenced by the amino acid composition of the protein, the level of energy in the diet, strain of birds, environmental temperatures, and perhaps other factors not known at this time. Such factors are probably responsible for a great deal of the variation in protein requirements as suggested by the various research workers.

With corn-soybean oil meal type laying rations, protein levels of 16 to 18 percent of the diet are most commonly employed under practical conditions. The most limiting amino acid in such diets is generally considered to be methionine. It has been reported by Scott (1960) that methionine supplementation of 16 and 18 percent protein diets resulted in improved egg production and feed efficiency.

Work to be reported in this thesis with an 11 percent level of protein has indicated that methionine is not the first limiting amino acid but that this role is taken over by lysine. This work has also indicated that amino acids other than methionine and lysine are limiting in high energy, low protein diets.

The objectives of this work were:

1. To study the effect upon laying hen performance from methionine and lysine supplementation of 16 percent protein diets containing two levels of energy.
2. To determine the limiting amino acids for egg production in low protein diets having calorie-protein ratios similar to a 16 percent protein diet.
3. To determine the limiting amino acids for egg production in a high energy, 11 percent protein diet.

REVIEW OF LITERATURE

Protein and Amino Acid Requirements

Protein

The protein requirement for egg production has been studied extensively in the past. In a review, Heuser (1941) stated that in order to promote satisfactory egg production, to maintain body weight, and to support good hatchability and egg size, the ration should provide the amounts of protein which would ordinarily be supplied by a diet containing 15 to 16 percent protein. Since that time, research results on the laying hen's protein requirement have been quite variable. Those reports which indicate that lower protein levels for laying hens might be justified will be considered first.

Thornton and co-workers (1956) compared pullet performance on four different all vegetable basal rations of 11, 13, 15 and 17 percent protein. There were no statistically significant differences in egg production between these four basal rations. Egg size was reduced with a reduction in dietary protein; however, these differences became less as the age of the hens increased. Egg quality, as measured by Haugh Units, and shell thickness were not affected by the different levels of protein. Gain in body weight over the experimental period was slightly lower in those hens fed the 11 percent protein ration. Feed efficiency tended to vary with egg production as expected; therefore, no large differences in results were observed between rations of different protein levels. In a similar report, Thornton *et al.* (1957) indicated that the required protein level for egg production in caged layers may be as low as 13 percent.

Miller, Sunde and Elvehjem (1957) reported good egg production with diets containing 12.5 to 13 percent dietary protein. The level of dietary energy or protein in the maternal diet did not affect the growth rate of the chicks.

MacIntyre (1958) compared protein levels of 12.9 to 14.2 percent with normal levels of 15.7 to 17.5 percent. The low protein rations resulted in increased feed consumption and decreased feed efficiency. Egg production data suggested that the protein was marginal in the low protein diets and indicated that with proper amino acid balance, protein levels as low as 12 percent might be adequate for laying hens.

Similarly, Adams _et al_. (1958) presented data from a preliminary test indicating that a corn-soybean oil meal ration containing 12 percent protein gave as satisfactory egg production as higher protein levels when all known vitamins were added to the ration. Data on various levels of protein fed to Leghorn layers in floor pens indicated that the optimum level of protein in a corn-soybean oil meal ration appeared to be between 12 and 14 percent when the usual vitamins were added. These workers also reported that certain vitamin additions might be beneficial when low levels of soybean oil meal were fed.

Johnson and Fisher (1959) compared a 15.7 percent protein diet to diets containing 10.4 and 11.3 percent protein. The 10.4 percent protein diet, containing wheat, supported egg production equal to that obtained on the 15.7 percent ration. Egg size on the low protein diets was significantly smaller but body weights were equal at the end of the experimental period. The 11.3 percent protein diet, containing corn, was inferior in all respects to the low protein wheat diet and the 15.7 percent protein ration. Reasons given by the authors involved the relationship of non-essential to essential amino acids or that differences occurred in the protein digestibility of the two rations. It was concluded that the good results obtained with the 10.4 percent protein diet confirmed under practical conditions the previous estimation of the minimal essential amino acid requirements of Johnson and Fisher (1958).

Frank and Waibel (1960) fed caged White Leghorn hens diets containing 10.2, 12.4, 14.9, 19.9 and 29.9 percent protein. With low and high energy diets, 12.4 and 14.9 percent protein, respectively, allowed normal egg production. The protein level did not appear to affect body fat or serum cholesterol levels.

Thornton and Whittet (1959) and (1960) reported on the protein requirement as affected by management, genetic background and dietary energy level. Four different protein levels were observed (11, 13, 15 and 17 percent). Comparable egg production rates were obtained at the three higher levels under all conditions of the experiment. The 11 percent protein diet was similar to the higher levels only when the dietary energy level was reduced.

Information discussed to this point has favored the theory that the protein requirement of the laying hen is less than 15 percent of the diet. Research reports supporting higher protein requirements will now be presented.

Reid, Quisenberry and Couch (1951) reported that egg production of SCWL pullets was reduced when the protein content of the basal rations was lowered from 18 percent to 15 percent or to 13 percent. The diets were essentially corn-soybean oil meal rations.

Berg and Bearse (1957) studied the effects of diets containing 14, 16 and 18 percent protein. With a high energy diet, production was depressed with the 14 percent level of protein. Protein levels of 16 and 18 percent promoted essentially the same rate of lay, although there was a tendency for higher production with an 18 percent diet.

A protein effect was not as evident with a low energy as it was with a high energy diet. There was a tendency, however, for the rate of lay to decrease as the level of protein increased. The rates of lay on the high energy, 18 percent protein and the low energy, 14 percent protein diets were the same. With 16 percent protein the energy level did not affect production rate.

Milton and Ingram (1957) studied protein requirements as affected by various factors. In a comparison of 12, 14, 16 and 18 percent protein, the highest level gave the best results under high temperatures. When old hens and pullets were compared, the hens showed slightly higher egg production with 16 and 18 percent protein. Pullets produced as well on 14 percent protein as on higher levels. The 12 percent ration proved inadequate. Heywang _et al_. (1955) reported that no increase in egg production would occur if the protein level in the diet of laying chickens was greater than 15.0 percent during hot weather. Levels of 11.5, 13.0, 15.0, 16.5, 18.0 and 19.3 percent protein were employed in their research.

Hochreich *et al.* (1958) presented results with SCWL pullets indicating that 17 percent protein in the feed was required to maintain maximum egg production and feed efficiency when the diet contained 950 Calories of productive energy per pound. The protein level did not significantly affect fertility, hatchability, mortality or the body weight of hens. These research workers were using diets containing 15.7, 17.0 and 18.3 percent protein.

Quisenberry and Bradley (1962a) reported that hen-day egg production, egg weight and feed efficiency were significantly improved with each increase in protein from 13 through 15 and 17 percent. The higher level of dietary protein offered promise as a means of reducing the number of undersized eggs laid by pullets during the early stages of egg production. Overall change in body weight was not significantly influenced by the dietary protein level.

Gordon and co-workers (1962) fed protein levels of 11, 15, 19 and 23 percent and energy levels of 860, 980 and 1100 Calories of productive energy. Increasing the protein to 19 percent increased egg production, egg weight and feed efficiency with no improvement from 23 percent protein. Increasing the energy decreased feed consumption thereby improving feed efficiency. Energy had no effect on egg production or egg weight. At the lowest calorie level, 23 percent protein depressed egg weight while at higher calorie levels it had no such effect. A similar Calorie:protein interaction was found for feed efficiency and egg production.

With diets varying in milo, corn and protein levels Malik and

Quisenberry (1963) found that in comparison to 15 percent protein, 18 percent protein supported higher egg production, larger eggs and higher feed efficiency. The differences were significant.

Amino Acids

Studies with amino acid requirements for laying hens have been limited. Johnson and Fisher (1956) classified the amino acids as to their essentiality for the laying hen using a crystalline amino acid diet. They found arginine, glutamic acid, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine to be essential for egg production. With the exception of glutamic acid, omission of any one of the above amino acids resulted in immediate disruption of feed consumption and a 10 day pause in production when such incomplete diets were forced-fed for only 5 days. Although feed consumption was not affected by the omission of glutamic acid, normal egg production could not be maintained. The hens did not require glycine for egg production. In agreement with this was a report by Menge and Lillie (1956). They, too, found that glycine deficient diets did not affect egg production.

Johnson and Fisher (1958) studied the essential amino acid requirements of the laying hen. These workers used a free amino acid diet in their research work. After establishing the lysine requirement of the laying hen at 0.50 percent of the diet, other essential amino acids were included in the diet in the ratio of their content in whole egg. Their suggested requirements are presented in Table 2, page 25.

Ingram and co-workers (1951) reported that 0.38 percent methionine in the ration was adequate for the laying hen. The combined cystine and methionine requirement was reported to be not greater than 0.63 percent. Hatchability or the amino acid composition of eggs from hens fed methionine deficient diets was not affected. The estimated requirement for methionine by Leong and McGinnis (1952) was somewhat lower. They suggested that 0.28 percent methionine in the presence of 0.25 percent cystine was required by the laying hen for maximum egg production.

Factors Affecting the Requirements for Protein by Laying Hens

A review of literature on the laying hen's protein requirement reveals the wide variation which has been obtained in research results. This variation is probably due to many influencing factors. A number of experiments have been conducted to study these factors.

A linear relationship between energy levels and efficiency of feed utilization for egg production was demonstrated by Hill *et al.* (1954). This study prompted a great deal of research on the effects of energy levels on nutrient requirements. Berg and Bearse (1957) indicated a protein:energy relationship affecting rate of lay in their work. These workers demonstrated that the requirement for protein by the laying hen is dependent upon the energy content of the diet.

The effect of strain of birds, cage management, floor management and high and low dietary energy levels on the protein requirement was studied by Thornton and Whittet (1959) and (1960). Of these various factors, only the energy level affected the protein requirement. A level of 11 percent protein was insufficient when the dietary level of energy was high and sufficient when the energy level was reduced.

Similar results were reported by Frank and Waibel (1960). With low and high energy diets and 12.4 and 14.9 percent protein, respectively, adequate egg production was obtained. A energy:protein interaction was also reported by Gordon and co-workers (1962) at 23 percent protein. With 860 Calories of productive energy, 23 percent protein depressed egg weight while at higher Calorie levels it did not. A similar interaction of energy and protein level was found for feed efficiency and egg production.

Working with protein levels of 17 and 18 percent, Price _et al._ (1957) and McDaniel _et al._ (1957) reported no differences in egg production at various energy levels. However, feed efficiency was improved with each increase in energy level.

Contrasting the previous reports substantiating a protein:energy relationship was the research by Miller, Sunde and Elvehjem (1957). They found that increasing the productive energy levels from 640 to 1075 Calories per pound did not affect egg production at any of the protein levels fed. The protein levels included ranged from 12.5 to 20.9 percent. These researchers stated that the laying pullet can tolerate a wide Calorie:protein ratio without an effect upon egg production.

In addition to the protein:energy relationship, other factors which might affect the laying hen's requirement for protein have also been studied. Bray and Gesell (1961) reported on the effect of environmental temperature upon White Leghorn pullets fed graded levels of protein. Temperature extremes of 42°F. and 86°F. altered feed intake but did not appear to affect rate of lay provided protein intake remained above 12 grams per bird per day throughout the assay period.

The rate of decline in egg production of pullets fed a given suboptimal protein diet was greater at the higher temperature. An inverse relationship existed between temperature and egg production at suboptimal protein levels. It was concluded that temperature exerted its effect through feed intake, rather than by altering the absolute protein requirement for a given level of egg production.

Milton and Ingram (1957) fed isocaloric diets containing 12, 14, 16 and 18 percent protein. These diets were fed to hens housed in a room which was heated to approximately 90°F. each day and cooled to 70°F. at night. Under these conditions, an increase in production was obtained with each increase in protein up to 18 percent, the highest level used.

Contrary to the results obtained with high experimental temperatures by Milton and Ingram (1957) is the report by Heywang, Bird and Vavich (1955). These workers reported that no increase in egg production would result with protein levels in the laying hen's diet greater than 15.0 percent during hot weather and that the optimum level of protein in the diet of the laying chicken was the same during hot, cool and moderate weather. They point out, however, that these results might apply only to diets containing combinations of such good protein concentrates as sardine meal and casein.

Seasonal effects on protein requirements were studied by Bray and Morrissey (1962). When pullets were fed corn-soya diets containing 12 and 18 percent protein for a 42 week-assay period beginning in September and terminating in July, performance was comparable at the two protein levels except for the first 12-week and the last 6-week periods of the assay. During these periods performance was significantly lower for one of more criteria at the 12 percent protein level. These results indicate the necessity of considering the level of feed intake, maturity and physiological status of the bird, rate of lay and/or combinations of these factors in interpreting the results of experiments on the protein requirement of the laying hen.

Harms and Waldroup (1962b) suggest that strain and breed differences may account for some of the variation reported for laying hen protein requirements. In their experimental work, a significant strain: protein level interaction, as measured by rate of egg production, was observed. Each strain required approximately 20 grams of protein to produce an egg, and each strain made an attempt to overconsume on energy to meet this need. However, one strain could not completely compensate and had a requirement in excess of 13 percent protein while this level was adequate for the other strain.

Amino Acid Supplementation of Low Protein (8-14 percent) Laying Hen Diets

In view of the fact that the National Research Council (1960) has recommended 15 percent protein in the laying hen diet and that some research work has indicated the protein requirements to be 15 percent and higher, a number of experiments have been conducted to determine the limiting amino acids in low protein diets.

Reid and co-workers (1951) reported that methionine additions to rations containing 13 percent protein did not consistently improve egg production. Similarly Thornton *et al*. (1956) and Thornton *et al*. (1957) obtained no benefit as measured by egg production, feed efficiency or body weight from the addition of 0.2 percent DL-methionine, 0.1 percent L-lysine or a combination of these amino acids to all vegetable basal rations containing 11, 13, 15 and 17 percent protein. However, DL-methionine increased egg weight and reduced small egg incidence at all protein levels. L-lysine supplementation was effective in reducing incidence of small eggs at the two lower protein levels, but increased the incidence at the two higher levels. At the 11 and 13 percent protein level, the use of the two amino acids together was non-effective.

In work by Bray (1960), significant responses were obtained with the addition of 0.16 percent DL-tryptophan and 0.43 percent L-lysine HCl to a 10 percent protein corn-soya diet. Methionine alone did not significantly increase egg production although the increase was of a magnitude of 12 percent.

In a later report, Bray and Garlich (1960) compared the performance of laying pullets fed a ration containing 9 percent protein in which corn and soybean oil meal were the only sources of protein, and the same diet supplemented with the amino acids needed to meet the minimal requirements suggested by Johnson and Fisher (1958). A corn-soya ration containing 16 percent protein served as the positive control. The supplemental amino acids were tryptophan, lysine, methionine, isoleucine and valine. These amino acids increased egg production and nitrogen retention significantly. Egg production, however, did not equal that obtained with the 16 percent protein control. Supplementation of the low protein diet improved weight gain although not equal to that of the control group.

In further reports by Bray and Morrisey (1962), results of amino acid supplementation of a 60:40 blend of corn and soybean protein were presented. This diet contained 9 percent protein. Additions of methionine, tryptophan, lysine, isoleucine and valine were made on the basis of the egg protein ratio standard presented by Johnson and Fisher (1958). There was no response in egg production and body weight when the diet was supplemented either with 1.5 percent protein from glutamic acid or with methionine, tryptophan and lysine. Supplementations of methionine, tryptophan, lysine, isoleucine, valine or the latter combination of amino acids plus 1.5 percent protein from a 60:40 blend of corn and soybean protein each increased rate of lay and body weight. When methionine, tryptophan, lysine, isoleucine and valine were fed in a factorial experiment, isoleucine and valine improved performance only in the presence of methionine, tryptophan and lysine, isoleucine being more effective than valine in this respect.

Isoleucine and valine were incorporated into the basal diet used to factor methionine, tryptophan and lysine. Lysine, tryptophan and methionine supplementation resulted in a highly significant, significant, and a non-significant increase in egg production, respectively.

Waldroup and Harms (1961) supplemented corn-soybean meal type diets containing 3 percent fish meal with amino acids. These diets were fed to commercial, egg production type, laying pullets. The results indicated that lysine was the first limiting amino acid in an 11 percent protein diet with methionine being second. Supplementing the diets with these limiting amino acids resulted in improved feed utilization and increased rate of egg production in most of the experiments. Similar results were presented by Harms and Waldroup (1962a) in a later report.

Contrary to the previous reports, when added singly to an 11 percent protein ration (corn-soybean oil meal), methionine but not lysine improved egg production and feed efficiency. These results were presented by Yates and Schaible (1961). The combination of lysine and methionine was of no added benefit. Hens receiving supplemental methionine gained less body weight than any of the other groups. Egg size was larger with the 16 percent protein control ration and 11 percent protein ration containing both methionine and lysine than with the 11 percent protein unsupplemented or singly supplemented rations.

Bradley and Quisenberry (1961) reported significant increases in production when a 14 percent protein diet was supplemented with amino acids. These workers added 1.057 pounds of lysine, or 2 pounds of methionine, or a combination at the same levels per ton of ration. The lysine supplementation produced greater increases in production than methionine and reduced the feed required per unit of eggs. Egg size was generally larger for the supplemented groups, but the increases were too small to be of significance.

In a later report by Quisenberry and Bradley (1962b), methionine and lysine supplementation in a diet containing 14 percent protein had no effect except to slightly increase body weight. Supplementation of a 13 percent protein milo-corn-soy diet with 1 pound of lysine or 2 pounds of methionine per ton or the combination increased egg production. Egg size was lower and feed efficiency was inferior on the supplemented 13 percent protein diet. The supplemented and unsupplemented 14 percent protein diets were inferior in these respects to the unsupplemented 16 and 18 percent protein diets.

In work by Stangeland and Carlson (1961), the addition of methionine alone to an 11 percent protein corn-soybean meal diet did not affect egg production whereas the combination of methionine and lysine consistently improved egg production and feed efficiency. However, egg production and feed efficiency were superior for the positive control group which received a 16 percent protein diet.

Similar results were reported by Waibel and Johnson (1961). The addition of methionine and lysine to a 10 percent protein diet improved egg production somewhat but not equal to that of the 16 percent protein control. Methionine alone was not effective. Hens receiving the further addition of 0.04 percent DL-tryptophan showed increased egg production of 3.2 percent while 0.15 percent added DL-valine depressed egg production.

Romoser *et al.* (1962) added methionine-hydroxy-analog (MHA) and L-lysine HCl both singularly and in combination to 11 percent protein diets at energy levels of 860, 980 and 1100 Calories of productive energy per pound. The diets were formulated conventionally and also by maintaining the ratio of corn to soybean oil meal of a 15 percent protein diet by dilution of the protein with glucose and tallow. Added MHA prevented the depressing effects of added energy. In addition, supplementing the diet with MHA independently, increased egg weight, egg production and feed efficiency. These effects were greatest when the corn-soy ratio was that of a 15 percent protein diet. This narrow corn-soy ratio resulted in an increase in rate of lay, weight gain, egg weight and feed efficiency indicating an improved amino acid balance. Lysine supplementation improved feed efficiency and egg weight but it was not possible to determine whether methionine or lysine was the first limiting amino acid.

With an experimental diet consisting of 80 percent corn as the sole source of protein, Fitzsimmons and co-workers (1963) reported that the addition of supplemental amino acids to bring the dietary total to 125 percent of the minimum estimates of Johnson and Fisher (1958) was required to maintain body weight, egg production and egg size. Similar effects were obtained with the addition of 7.68 percent isolated soybean protein which increased the total dietary protein to 13.1 percent.

<u>Amino Acid Supplementation of High Protein Laying Hen Diets</u>

One could conclude that methionine and lysine are the first and second limiting amino acids, respectively, in corn-soybean oil meal laying hen diets containing 15 to 16 percent protein. Consequently, a considerable amount of research has been directed toward the supplementation of high protein laying hen diets with these amino acids. One of the first experiments with methionine supplementation was presented by Taylor and Russell (1943). These workers fed methionine by capsule at the rate of 0.7 mg. per hen per day in an attempt to shorten the molt period and to stimulate more egg production. The supplementation did not affect either the molt period or egg production.

Reid, Quisenberry and Couch (1951) reported that a reduction of the protein content of corn-soybean rations for SCWL pullets from 18 percent to 15 percent or to 13 percent resulted in reduced egg production. Addition of methionine to the lower protein rations did not consistently improve egg production.

In work with New Hampshire pullets, Mehring and co-workers (1954) added 0.0847 percent DL-methionine to a 16 percent protein diet containing from 0.25 to 0.3127 percent methionine and 0.2592 to 0.2668 percent cystine. The addition of methionine to the corn-soybean diet had no statistically significant effect on egg production, feed efficiency or the gain in live weight. The addition did beneficially affect the hatchability of fertile eggs; however, the differences due to the methionine were of borderline significance.

Heywang (1956) used diets containing 16.9 percent and 15.5 percent protein and SCWL pullets in two experiments. In one, the basal diet contained soybean oil meal as the only source of protein; whereas, the other contained 2½ parts of fish meal in place of 3½ parts of the soybean oil meal. These diets were supplemented with two levels of DL-methionine. The addition of methionine had little, if any, effect on egg production, diet consumption per dozen eggs, egg weight, shell thickness, hatchability or pullet weight. The basal diet in the first experiment contained about 0.28 percent L-methionine and 0.56 percent L-cystine and in the second experiment about 0.27-0.29 percent L-methionine and 0.49 percent L-cystine.

Yates and Schaible (1961) reported that egg production was slightly less when higher levels of lysine (0.5 percent) and methionine (0.25 percent) were fed singly and in combination in a 17 percent protein ration. These hens were in floor pens.

Similar results were obtained in work by Bradley and Quisenberry (1961). In their research, supplementation of 16 and 18 percent protein diets with 1.057 pounds of lysine and two pounds of methionine per ton of feed individually and in combination resulted in non-significant depressions in egg production.

In research by Gordon and co-workers (1962), addition of 0.1 percent of MHA to a 15 percent protein diet improved egg production, egg weight and feed efficiency such that it was equal to that obtained with a 19 percent protein unsupplemented diet and nearly equal to the results obtained from a 19 percent protein diet supplemented with MHA. There appeared to be a beneficial effect from the addition of MHA even at the relatively high protein level of 19 percent.

Scott (1960) presented an interesting approach to amino acid nutrition of the laying hen by comparing the quantity of amino acids provided by a typical corn-soy type laying diet with the total daily requirement for egg production and tissue replacement on the basis of calculations. Such a comparison illustrated that methionine was limiting in a 16 percent protein diet. On the basis of experimental work, Scott concluded that when the energy content of the diet was adequate, the addition of methionine to a corn-soybean laying ration increased egg production, improved efficiency of feed utilization, increased egg size and improved efficiency of energy utilization.

Data were presented by Harms, Douglas and Waldroup (1962) for three laying hen experiments. Supplementing corn-soybean type diets containing from 13 to 16.7 percent protein with 0.075 percent MHA-calcium resulted in improving laying performances in two of the three experiments. The energy content of the diets appeared to be a factor in controlling the response from the supplementation.

Similar evidence was presented by Heywang and co-workers (1963). These researchers added supplemental methionine as 0.05 percent MHA to 16 percent protein diets with 1350, 1450 and 1550 Calories of metabolizable energy per pound in two experiments. In a third experiment, diets with no supplementation and 1550 Calories of metabolizable energy per pound with additions of 0, 0.05 and 0.10 percent of MHA were fed.

Supplementation of the 1350 Calorie diet did not increase egg production or decrease the pounds of feed consumed per dozen eggs laid. The results with the 1450 Calorie diet were inconsistent. However, in all three experiments the inclusion of 0.05 percent MHA in the 1550

Calorie diet resulted in a slight improvement in egg production and a corresponding increase in feed conversion. Some of the differences were not statistically significant. In the third experiment, the inclusion of 0.10 percent MHA in the 1550 Calorie diet resulted in further improvement in egg production and feed efficiency.

A level of 0.41-0.44 percent methionine plus cystine/therm metabolizable energy per pound appeared to be adequate for egg production under the conditions of these studies. Maintenance of live weight on the 1450 and 1550 Calorie, but not on the 1350 Calorie diets, was a little better when these diets contained the amino acid supplementation. The MHA had little effect at any caloric level on egg weight, hatchability of fertile eggs or mortality.

Work by Olson et al. (1962) indicated that the addition of DL-methionine to a 16 percent protein laying diet slightly improved the rate of lay and feed efficiency, although the differences were not significant. The addition of MHA-calcium to diets containing from 10 to 16 percent protein slightly decreased the rate of lay and feeding efficiency. A level of 0.1 percent DL-methionine and MHA-calcium was used in this work. The diets contained from 1268 to 1442 Calories of metabolizable energy per pound. A hen-day intake of 16 grams of good quality protein appeared to be adequate to support a production rate of 70 percent under the conditions of this research.

EXPERIMENTAL PROCEDURE

The research work reported here was conducted over a period of two years. Regional Control Single Comb White Leghorn pullets which were obtained from the North Central Regional Poultry Breeding Laboratory, U.S.D.A., Lafayette, Indiana were used. The pullets were reared on a green range and were fed practical type starter and grower rations. At housing time, the obvious culls and immature birds were eliminated and the balance of the pullets were vaccinated for Newcastle Disease with a killed virus vaccine and leg banded. The pullets which were to be allotted to floor pens were also debeaked. A standard corn-soybean oil meal laying ration was fed after housing until the pullets were placed on their experimental regime.

On the basis of individual records, those pullets not producing consistently during the month prior to the beginning of the experiments were removed. The birds were individually weighed at the beginning of the experiments, at three month intervals, and again at the end of the experiments. Body weight change was the difference in initial body weight and final weight calculated as the average of the two replicate groups of each treatment in the cold-wall environment and as average of the individuals in each treatment in the warm-wall environment.

To determine dietary effects upon egg weight and interior quality, eggs were saved for a period of two days and four days in the cold and warm-wall environments, respectively. Interior quality was obtained by determining Haugh Units (Haugh, 1937).

Table 1. Composition of Laying Hen Basal Diets.

Ingredient	220	221	222	223	224
	%	%	%	%	%
Ground yellow corn	85.00	66.00	56.10	72.50	49.50
Soybean oil meal (44%)	5.00	19.00	3.30	18.00	14.25
Oat mill feed	----	----	28.00	----	----
Alpha cellulose[1]	----	----	----	----	24.75
Yellow grease	0.50	5.50	----	----	2.00
Isolated soybean protein[2]	----	----	2.60	----	----
Dehydrated alfalfa meal (17%)	2.00	2.00	2.00	2.00	2.00
Dicalcium phosphate	2.00	2.00	2.00	2.00	2.00
Limestone	5.00	5.00	5.00	5.00	5.00
Salt mix[3]	0.50	0.50	0.50	0.50	0.50
	Added per pound of diet				
Vitamin A, I. U.	1080.0	1080.0	1080.0	1080.0	1080.0
Vitamin D_3, I. C. U.	375.0	375.0	375.0	375.0	375.0
Vitamin E, I. U.	5.0	5.0	5.0	5.0	5.0
Vitamin K, mg.	1.0	1.0	1.0	1.0	1.0
Choline, mg.	292.0	170.7	292.0	170.7	170.7
Niacin, mg.	15.0	13.2	15.0	13.2	13.2
Pantothenic acid, mg.	2.5	2.2	2.5	2.2	2.2
Riboflavin, mg.	2.5	2.2	2.2	2.2	2.2
Cyanocobalamin, mcg.	4.5	4.5	4.5	4.5	4.5
Protein, %	11.2	15.9	10.3	15.9	11.2
Metabolizable energy, Cal./lb.	1421	1414	1001	1340	1004
Calorie-protein ratio	127	89	97	84	89

1 Solka-Floc, a cellulose product of Brown Company, Boston, Mass.

2 Promine R Protein manufactured by Central Soya Co., Inc., Chicago, Illinois

3 Containing 0.455 % manganese, 0.011% iodine, 0.01% cobalt, 0.165% iron, 0.048% copper, 0.30% sulfur, and 97.0% sodium chloride.

The composition of the experimental diets used is given in Table 1, page 23. The diets were essentially corn-soybean type rations with the exception of diets 222 and 224 which contained low energy ingredients in order to reduce their caloric content. Diet 220 was a low protein diet containing approximately 11 percent protein and was isocaloric with diet 221 which contained 16 percent protein. Diets 222 and 224 were low protein, low energy diets containing approximately 10 and 11 percent protein, respectively, and having Calorie-protein and corn to soybean oil meal ratios approximately the same as diet 221. Diet 223 contained approximately 16 percent protein and was formulated to contain less energy than diet 221. Feed and water were provided *ad libitum* in all of the experiments.

Methionine and lysine additions to the high protein diets were made on the basis of the most commonly used levels in other research reports. Additions of these amino acids to the low protein diets were made at a level which would bring their content approximately equivalent to that of the high protein diets. The other amino acids were added to diets 222 and 224 in quantities necessary to make the dietary content approximately equivalent to 125 percent of the levels suggested by Johnson and Fisher (1958). The suggested requirements of Johnson and Fisher (1958) and the National Research Council (1960) and the amino acid composition of the low protein basal diets are given in Table 2, page 25.

Duration of the experiments varied from 5 to 7 months. The research work was conducted under two environmental conditions and

Table 2. The Calculated Amino Acid Composition of the Low Protein Basal Diets and the Suggested Requirements.

	Requirements					
	Johnson & Fisher 1958	NRC 1960	Johnson & Fisher X 1.25	Basal Diet Content[1] 220	222	224
	%	%	%	%	%	%
Arginine	0.74		0.93	0.54	0.57	0.68
Cystine	0.16	0.25	0.20	0.11	0.09	0.13
Glycine	0.50		0.63	0.50	0.42	0.26
Histidine	0.18		0.23	0.21	0.17	0.24
Isoleucine	0.50	0.50	0.63	0.51	0.48	0.59
Leucine	0.68	1.20	0.85	1.00	0.82	0.97
Lysine	0.50	0.50	0.63	0.31	0.39	0.52
Methionine	0.24	0.28	0.30	0.17	0.15	0.15
Phenylalanine	0.42		0.53	0.49	0.42	0.53
Threonine	0.36	0.40	0.45	0.33	0.33	0.42
Tryptophan	0.12	0.15	0.15	0.11	0.12	0.14
Tyrosine	0.30		0.38	0.45	0.36	0.42
Valine	0.54		0.68	0.50	0.44	0.56

[1] Calculated on the basis of the amino content of ingredients as given in the Merck & Company bulletin entitled "Analysis of Feedstuffs" (1961).

pertinent procedures for each environment will be presented separately.

Duncan's multiple range analysis (Steel and Torrie, 1960) was used to separate the treatment means.

Cold-Wall Environment

Experiments 1a and 1b were conducted in successive years in a gravity ventilated, cold-wall, wooden frame building. This building contained sixteen 10 ft. by 12 ft. floor pens in two rows with eight adjacent pens per row and the rows separated by an alley. Straw, which was periodically replaced, was used for litter.

At approximately 5 months of age, forty pullets were randomly allotted to each floor pen. Four males were also placed in each pen. Each pen made up a replicate and each treatment consisted of two replicates.

Hen-day egg production and feed efficiency were calculated for each replicate group and the data presented as the means of the two replicates in each treatment. Feed consumption was corrected by subtracting nine pounds per male per month from the total to adjust for the consumption of the males.

For hatchability studies, eggs were saved during the fifth month of experiment 1a and the fourth month of experiment 1b. The fertility of the eggs set and hatchability of fertile eggs were determined and the means of the two replicates in each treatment calculated.

For statistical analysis of egg production, feed efficiency, fertility and hatchability, replicate means were used. To analyze statistically the egg weight and Haugh Unit data, replicate means for each day's eggs were used.

Warm-Wall Environment

Experiments 2a and 3a were conducted during the first year of the research work and experiments 2b and 3b the following year. The experiments were carried out in a windowless, forced-air ventilated, and well-insulated house of wooden frame construction.

At approximately $5\frac{1}{2}$ months of age, the pullets were randomly allotted to individual cages. In experiments 2a and 2b each treatment consisted of approximately 15 birds in adjacent individual cages with one feed trough per treatment group.

In experiments 3a and 3b there were six treatment groups with each treatment being made up of 10 birds in individual cages. The pullets were allotted to treatments by stratified randomization on the basis of body weight, and as a result birds receiving the six different treatments were randomly dispersed in the sixty adjacent cages. These cages had individual feeders and waterers.

Hen-day egg production was determined for the individual birds and calculated as the treatment means. Feed efficiency for experiments 2a and 2b was calculated for the entire treatment group. In experiments 3a and 3b the feed efficiency was determined for each individual bird and the mean of the treatment group calculated.

For statistical analysis, individual data for hen-day egg production and body weight changes were used in experiments 2a, 2b, 3a and 3b. For analysis of egg weight and Haugh Unit data, the treatment means for each day's eggs were used. In experiments 3a and 3b, the feed efficiency for each individual was used. Statistical analysis of feed efficiency for experiments 2a and 2b was not possible due to the lack of replicate groups or individual data.

RESULTS AND DISCUSSION

Experiment 1a

The egg production and feed efficiency data for experiment 1a are presented in Table 3, page 30. The addition of methionine and lysine singly or in combination to the 16 percent protein diet did not beneficially affect egg production or feed efficiency. Both methionine and lysine when added singly appeared to depress egg production although the differences were not significant statistically.

The addition of lysine to the 11 percent protein diet, which contained 0.2 percent added DL-methionine, improved egg production slightly and feed efficiency by 0.63 of a pound per dozen eggs. However, neither of the responses was of statistical significance.

The final weight of the hens and the changes in body weight are given in Table 4, page 31. All of the groups receiving the 16 percent protein diet gained body weight during the experimental period while those on the low protein regime lost weight. Changes in body weight for the low protein groups were significantly different from those of the high protein groups. Differences between treatments receiving the same level of protein were not of statistical significance.

The addition of methionine and lysine to the 16 percent protein diet, as shown in Table 5, page 33, did not affect egg weight; whereas, egg weight was significantly increased when lysine was added to the 11 percent protein diet. The egg weight with the methionine supplemented 11 percent protein diet was significantly less than that from any of

Table 3. Hen-day Egg Production and Feed Efficiency of Hens Fed High and Low Protein Diets Supplemented With Methionine and Lysine for Eight Months.

Experiment 1a

Treatment[1]	Egg Production	Feed Efficiency
	%	lbs./doz.
1. 16% protein[2]	68.2b[4]	4.41a
2. As 1 + 0.15% DL-methionine	65.7b	4.60a
3. As 1 + 0.1% L-lysine	63.9ab	4.57a
4. As 3 + 0.15% DL-methionine	67.8b	4.55a
5. 11% protein[3] + 0.2% DL-methionine	50.8a	6.12b
6. As 5 + 0.3% L-lysine	53.1a	5.49b

[1] The data presented for each treatment are the average of two replicates with each replicate consisting of approximately 40 hens.

[2] Basal Diet 221, composition given in Table 1, page 23.

[3] Basal Diet 220, composition given in Table 1, page 23.

[4] Values having different subscripts are significantly different at the 0.05 level of probability.

Table 4. Body Weight Maintenance of Hens Fed High and Low Protein Diets Supplemented with Methionine and Lysine for Eight Months.

Experiment 1a

Treatment	Body Weight Change	Final Weight
	lbs./hen	lbs./hen
1. 16% protein[1]	0.11b[3]	4.23
2. As 1 + 0.15% DL-methionine	0.31b	4.35
3. As 1 + 0.1% L-lysine	0.22b	4.30
4. As 3 + 0.15% DL-methionine	0.21b	4.42
5. 11% protein[2] + 0.2% DL-methionine	-0.28a	3.94
6. As 5 + 0.3% L-lysine	-0.44a	3.75

1 Basal Diet 221

2 Basal Diet 220

3 Values having different subscripts are significantly different at the 0.05 level of probability.

the groups receiving the higher protein diets. The addition of lysine to the 16 percent protein diet resulted in a statistically significant increase in Haugh Units. This effect was not demonstrated with the addition of lysine to the low protein diet. Haugh Unit measurements were significantly higher for the low protein groups as compared to the 16 percent protein control treatment.

Neither protein level nor amino acid supplementation had any effect upon fertility. These data are shown in Table 6, page 34. Hatchability of fertile eggs was variable with the only significant difference being between treatment 6 and treatments 1, 3 and 4.

Experiment 1b

In this experiment, Table 7, page 36, egg production was considerably lower with the 11 percent protein diet as compared with treatment groups receiving 16 percent protein. Due to the within treatment variation however, the differences were not significant statistically. As in experiment 1a, the addition of methionine to Basal Diet 221 had no beneficial effect and again exhibited a non-significant, depressing effect on both egg production and feed efficiency.

The addition of methionine and a combination of methionine and lysine to Basal Diet 223, a lower energy diet than Basal Diet 221, did not result in an increase in egg production or improvement in feed efficiency. Although there was considerable variation in feed efficiency between the groups receiving 16 percent protein diets, none of the differences were of statistical significance.

The effect of the dietary regimes upon the body weight of the

Table 5. Weight and Haugh Units of Eggs From Hens Fed High and Low Protein Diets Supplemented with Methionine and Lysine

Experiment 1a

Treatment[1]	Egg Weight	Haugh Units
	gm./egg	
1. 16% protein[2]	58bc[4]	70a
2. As 1 + 0.15% DL-methionine	58bc	71ab
3. As 1 + 0.1% L-lysine	57b	74cd
4. As 3 + 0.15% DL-methionine	59c	73bc
5. 11% protein[3] + 0.2% DL-methionine	55a	76d
6. As 5 + 0.3% L-lysine	57b	75cd

[1] The data given are the averages of two days production after six months on the experimental regime.

[2] Basal Diet 221

[3] Basal Diet 220

[4] Values having different subscripts are significantly different at the 0.05 level of probability.

Table 6. Fertility and Hatchability of Eggs From Hens Fed High and Low Protein Diets Supplemented With Methionine and Lysine.

Experiment 1a

Treatment[1]	Total Number of Eggs Incubated	Fertility	Hatchability[2]
		%	%
1. 16% protein[3]	431	93.8a[5]	85.6a
2. As 1 + 0.15% DL-methionine	433	95.1a	88.4ab
3. As 1 + 0.1% L-lysine	450	93.1a	86.5a
4. As 3 + 0.15% DL-methionine	417	96.2a	86.6a
5. 11% protein[4] + 0.2% DL-methionine	312	95.1a	89.2ab
6. As 5 + 0.3% L-lysine	326	96.2a	93.0b

1 These determinations were made during the fourth month of the experiment.

2 Hatchability of fertile eggs.

3 Basal Diet 221.

4 Basal Diet 220.

5 Values having different subscripts are significantly different at the 0.05 level of probability.

hens during the experimental period is shown in Table 8, page 37. The group receiving the low protein diet lost weight during the experiment; whereas, all of the high protein groups gained weight. Neither the addition of methionine to Basal Diet 221 nor the addition of methionine or a combination of methionine and lysine to Basal Diet 223 affected the change in body weight significantly. The hens in treatments 2 and 3, receiving the higher energy 16 percent protein diet, gained more weight than those hens receiving the lower energy 16 percent protein diet, treatments 4, 5 and 6. The difference between the body weight gain of hens in treatment 3 and the gain of hens in treatments 4, 5 and 6 was statistically significant.

As in experiment 1a, hens receiving the low protein diet produced significantly smaller eggs with significantly higher Haugh Units than hens receiving higher protein diets. These data are presented in Table 9, page 38. There were no differences in egg weight or Haugh Units between the two groups receiving the basal 16 percent protein diets. Eggs from treatments 5 and 6 were significantly smaller than those of treatment 3, however, this difference did not appear to be meaningful. Neither energy level nor amino acid supplementation had an effect on the Haugh Units of eggs from groups receiving 16 percent protein.

As shown in Table 10, page 39, there were no significant differences between treatment groups for fertility or hatchability of fertile eggs.

Under the conditions prevailing in experiments 1a and 1b, the

Table 7. Hen-day Egg Production and Feed Efficiency of Hens Fed a Low Protein Diet and High Protein Diets of Two Energy Levels, Supplemented With Methionine and Lysine for Seven Months.

Experiment 1b

Treatment[1]	Egg Production	Feed Efficiency
	%	lbs./doz.
1. 11% protein[2]	51.9a[5]	6.60b
2. 16% protein (1414 M.E. Cal./lb.)[3]	63.8a	5.12a
3. As 2 + 0.05% DL-methionine	59.8a	5.72ab
4. 16% protein (1340 M.E. Cal./lb.)[4]	61.2a	5.36ab
5. As 4 + 0.05% DL-methionine	59.9a	5.42ab
6. As 5 + 0.1% L-lysine	61.7a	5.46ab

[1] The data presented for each treatment are the average of two replicates with each replicate consisting of approximately 40 hens.

[2] Basal Diet 220.

[3] Basal Diet 221.

[4] Basal Diet 223, composition given in Table 1, page 23.

[5] Values having different subscripts are significantly different at the 0.05 level of probability.

Table 8. Body Weight Maintenance of Hens Fed a Low Protein Diet and High Protein Diets of Two Energy Levels, Supplemented With Methionine and Lysine for Seven Months.

Experiment 1b

Treatment	Body Weight Change	Final Weight
	lbs./hen	lbs./hen
1. 11% protein[1]	-0.10a[4]	4.00
2. 16% protein (1414 M.E. Cal./lb.)[2]	0.50bc	4.60
3. As 2 + 0.05% DL-methionine	0.65c	4.65
4. 16% protein (1340 M.E. Cal./lb.)[3]	0.30b	4.35
5. As 4 + 0.1% DL-methionine	0.25b	4.40
6. As 5 + 0.1% L-lysine	0.30b	4.45

[1] Basal Diet 220.

[2] Basal Diet 221.

[3] Basal Diet 223.

[4] Values having different subscripts are significantly different at the 0.05 level of probability.

Table 9. Weight and Haugh Units of Eggs From Hens Fed a Low Protein Diet and High Protein Diets of Two Energy Levels, Supplemented With Methionine and Lysine.

Experiment 1b

Treatment[1]	Egg Weight	Haugh Units
	gms./egg	
1. 11% protein[2]	55a[5]	73b
2. 16% protein (1414 M.E. Cal./lb.)[3]	58bc	68a
3. As 2 + 0.05% DL-methionine	59c	67a
4. 16% protein (1340 M.E. Cal./lb.)[4]	58bc	69a
5. As 4 + 0.05% DL-methionine	57b	68a
6. As 5 + 0.1% L-lysine	57b	70ab

[1] The data given are the averages of two days production after seven months on the experimental regime.

[2] Basal Diet 220.

[3] Basal Diet 221.

[4] Basal Diet 223.

[5] Values having different subscripts are significantly different at the 0.05 level of probability.

Table 10. Fertility and Hatchability of Eggs From Hens Fed a Low Protein Diet and High Protein Diets of Two Energy Levels, Supplemented With Methionine and Lysine.

Experiment 1b

Treatment[1]	Total Number of Eggs Incubated	Fertility	Hatchability[2]
		%	%
1. 11% protein[3]	352	88.4a[6]	75.2a
2. 16% protein (1414 M.E. Cal./lb.)[4]	473	94.2a	78.7a
3. As 2 + 0.05% DL-methionine	403	94.4a	71.8a
4. 16% protein (1340 M.E. Cal./lb.)[5]	434	87.3a	68.7a
5. As 4 + 0.05% DL-methionine	438	93.6a	70.4a
6. As 5 + 0.1% L-lysine	373	95.9a	73.4a

[1] These determinations were made during the fourth month of the experiment.

[2] Hatchability of fertile eggs.

[3] Basal Diet 220.

[4] Basal Diet 221.

[5] Basal Diet 223.

[6] Values having different subscripts are significantly different at the 0.05 level of probability.

11 percent protein, high energy, corn-soybean oil meal diet was not adequate to maintain egg production, body weight and egg weight when compared to 16 percent protein diets. Neither fertility nor hatchability were affected by the protein levels used in these experiments. Similar to the results of Stangeland and Carlson (1961), the addition of a combination of methionine and lysine to the 11 percent protein diet improved performance slightly. However, the results were still inferior to those obtained with the 16 percent protein diets. This indicates that other amino acids might also be limiting in the low protein diet.

The lack of beneficial responses from the addition of methionine and lysine to the 16 percent protein diets suggests that the levels of these amino acids in the 16 percent protein diets are adequate for the rate of egg production obtained in this research work. On the basis of calculations, Basal Diets 221 and 223 contain 0.361 and 0.389 percent methionine plus cystine per megacalorie. This compares with the levels of 0.41-0.44 percent methionine plus cystine per megacalorie of metabolizable energy per pound found to be adequate for egg production by Heywang and co-workers (1963). The methionine plus cystine level as a percent of the diet was 0.51 and 0.52 percent for Basal Diets 221 and 223, respectively. This compares closely with the suggested requirement of 0.53 percent by the National Research Council (1960).

In a summary of recent research on the protein requirements of laying hens, Scott (1960) showed that the laying hen must consume about 18 grams of protein per day in order to achieve good egg production. As shown in Table 11, page 41, the best egg production in experiments 1a and 1b was obtained with protein intakes of 18 to 20 grams per hen per day thus closely agreeing with the report by Scott (1960). However, Scott (1960) has indicated that less protein would be required if methionine were added to the diet thereby increasing feed efficiency. The results from experiments 1a and 1b do not substantiate this.

Table 11. Grams of Protein Consumed Per Hen Per Day.

	Protein in Diet	Egg Production	Protein Per Day
	%	%	gms.
Experiment 1a			
Treatment 1	16	68.2	18.2
Treatment 5	11	50.8	12.9
Experiment 1b			
Treatment 1	11	51.9	14.2
Treatment 2	16	63.8	20.0
Treatment 4	16	61.2	19.9

Perhaps the addition of methionine did not reduce feed intake due to energy being a limiting factor. Heywang and co-workers (1963), when adding methionine to 16 percent protein diets containing 1350, 1450 and 1550 Calories of metabolizable energy per pound, found that there was no response with the 1350 Calorie diet, an inconsistent response with the 1450 Calorie diet but consistent improvement in both egg production and feed efficiency with the 1550 Calorie diet. Since the 16 percent protein diets in Experiments 1a and 1b contained only 1414 and 1340 Calories of metabolizable energy per pound this may explain the difference in results between this research and the work reported by Scott (1960).

Experiment 2a

The effect of methionine and lysine additions to Diet 220 upon the performance of laying hens under individually caged conditions was studied in experiment 2a. The data for egg production and feed efficiency are presented in Table 12, page 43. The addition of methionine to Basal Diet 220, the low protein, high energy diet, did not influence either egg production or feed efficiency. When a combination of methionine and lysine was added egg production was increased approximately fifteen percent and feed efficiency was improved substantially. This is in agreement with the work of Waldroup and Harms (1961) and Harms and Waldroup (1962a) but contrary to the findings of Yates and Schaible (1961).

Due to the variation in performance from hens on other treatments in this experiment, differences between treatments 1 and 2 and treatment 3 are not statistically significant. However, when the first three treatments of this experiment were analyzed separately,

Table 12. Hen-day Egg Production and Feed Efficiency of Hens Fed a High Protein and High and Low Energy, Low Protein Diets Supplemented With Amino Acids for Seven Months.

Experiment 2a

Treatment[1]	Egg Production	Feed Efficiency
	%	lbs./doz.
1. 11% protein (1421 M.E. Cal./lb.)[2]	39.1a[5]	6.21
2. As 1 + 0.2% DL-methionine	40.0a	6.34
3. As 2 + 0.3% L-lysine	54.4ab	5.34
4. 10% protein (1001 M.E. Cal./lb.)[3]	39.0a	7.73
5. As 4 + 0.25% DL-methionine	53.8ab	5.98
6. As 5 + 0.25% L-lysine	47.9ab	6.27
7. As 6 + 0.25% glycine	54.6ab	6.52
8. As 7 + 0.25% DL-valine	56.9b	6.16
9. As 8 + 0.35% L-arginine	55.7ab	6.47
10. As 9 + 0.4% DL-isoleucine	44.8a	7.37
11. As 10 + 0.04% DL-tryptophan	48.1ab	7.07
12. 16% protein[4]	61.3b	4.68

[1] Each treatment consisted of approximately 15 hens.

[2] Basal Diet 220, composition given in Table 1, page 23.

[3] Basal Diet 222, composition given in Table 1, page 23.

[4] Basal Diet 221, composition given in Table 1, page 23.

[5] Values having different subscripts are significantly different at the 0.05 level of probability.

the increase in egg production in treatment 3 was significant statistically. When compared to treatment 12, the 16 percent protein control, egg production from treatment 3 was considerably lower, though the difference was not significant. This difference indicates that amino acids other than methionine and lysine may be limiting for egg production in Diet 220.

Treatment 4, the low protein, low energy diet, allowed for egg production at the same rate as treatment 1, the higher energy diet, indicating that the energy content was not an influencing factor. The energy level did affect feed efficiency, however, with the hens in treatment 4 requiring approximately 1.5 pounds per dozen eggs more than the hens in treatment 1.

In contrast to the results with treatment 2, the addition of methionine to the 10 percent protein, low energy diet improved egg production and feed efficiency although the difference was not significant statistically. Egg production from treatment 5, though not statistically different, was lower than the egg production obtained with treatment 12. Further additions of amino acids to the low protein, low energy diet did not improve either egg production or feed efficiency. Additions of L-lysine and DL-isoleucine appeared to exhibit depressing effects as demonstrated by a reduction in egg production. This may have been due to an amino acid imbalance.

The egg weight, Haugh Unit and body weight data for experiment 2a are presented in Table 13, page 45. None of the dietary treatments

Table 13. Weight and Haugh Units of Eggs and Body Weight Maintenance of Hens Fed a High Protein and High and Low Energy, Low Protein Diets Supplemented With Amino Acids.

Experiment 2a

Treatment[1]	Egg Weight	Haugh Units	Body Weight Change	Final Weight
	gms/egg		lbs/hen	lbs/hen
1. 11% protein (1421 M.E. Cal./lb.)[2]	59a[5]	68ab	-0.10b	4.19
2. As 1 + 0.2% DL-methionine	60a	84c	-0.24ab	3.94
3. As 2 + 0.3% L-lysine	58a	78bc	-0.08b	4.29
4. 10% protein (1001 M.E. Cal./lb.)[3]	59a	80bc	-0.34ab	3.88
5. As 4 + 0.25% DL-methionine	58a	67a	-0.28ab	3.83
6. As 5 + 0.25% L-lysine	59a	79bc	-0.51a	3.44
7. As 6 + 0.25% glycine	58a	79bc	-0.29ab	3.92
8. As 7 + 0.25% DL-valine	61a	80bc	-0.23ab	4.02
9. As 8 + 0.35% L-arginine	59a	78bc	-0.13b	4.01
10. As 9 + 0.4% DL-isoleucine	59a	76bc	-0.24ab	4.16
11. As 10 + 0.04% DL-tryptophan	57a	74b	-0.34ab	3.85
12. 16% protein[4]	60a	75b	+0.36c	4.27

[1] The data for egg weight and Haugh Units are the averages of three days egg production during the sixth month of the experiment.

[2] Basal Diet 220.

[3] Basal Diet 222.

[4] Basal Diet 221.

[5] Values having different subscripts are significantly different at the 0.05 level of probability.

significantly affected egg weight. These results differed from those obtained in experiments 1a and 1b conducted in the cold-wall, litter floor environment in which protein level affected egg size. Haugh Unit measurements were quite variable with the differences being difficult to interpret on the basis of treatment effects.

Body weight maintenance was variable also with all of the groups receiving low protein diets losing weight; whereas, the high protein group gained weight. The difference was significant. The addition of lysine to the low energy diet seemed to depress body weight as it did egg production. There was no beneficial effect on weight gain by any of the amino acid additions.

<u>Experiment 2b</u>

Results from experiment 2b, Table 14, page 48, were erratic in comparison to experiment 2a. The addition of methionine to the high energy, 11 percent protein diet resulted in a depression in egg production but improvement in feed efficiency. This effect was not shown in experiment 2a or in previous research by Stangeland (1962). However, as in the previous experiments, the combined addition of methionine and lysine improved egg production and feed efficiency. Egg production and feed efficiency from this treatment were inferior, though not different statistically, to that obtained with the high protein group, treatment 12. Similar results were demonstrated in experiment 2a.

Finely ground cellulose was used as an energy diluent in Diet 224 making this diet very light and bulky. This resulted in considerable feed wastage and a diet not well accepted by the hens. Consequently, egg production was low and the quantity of feed required per dozen eggs very high.

Although egg production was low compared to experiment 2a, the effect of amino acid additions was quite similar to that obtained with Diet 222 in experiment 2a. Methionine again improved egg production and feed efficiency, whereas the addition of lysine resulted in an adverse effect upon egg production. Valine also depressed egg production and feed efficiency. However, none of the differences were statistically significant. No beneficial effect on egg production or feed efficiency, beyond that from methionine, was obtained from the addition of tryptophan, isoleucine, arginine, or a combination of the amino acids at a lower level.

The data for egg weight, Haugh Units, body weight change, and the final weight of the hens are given in Table 15, page 50. Again, considerable variation existed in this experiment. Egg weight was not affected by methionine or the combined methionine and lysine additions to the 11 percent protein high energy diet. Haugh Units were significantly lower with the methionine group than with treatments 1 and 3. This result differed from the previous experiments. Body weight was significantly less with the groups receiving the methionine additions than with treatment 1. From experiments 1a, 2a and this experiment it is apparent that the addition of methionine singly to Diet 220, the high energy, low protein diet, each time had an adverse effect upon body weight maintenance.

Table 14. Hen-day Egg Production and Feed Efficiency of Hens Fed a High Protein and High and Low Energy, Low Protein Diets Supplemented With Amino Acids for Six Months.

Experiment 2b

Treatment[1]	Egg Production	Feed Efficiency
	%	lbs./doz.
1. 11% protein (1421 M.E. Cal/lb.)[2]	51.6b[6]	5.71
2. As 1 + 0.2% DL-methionine	46.9b	5.24
3. As 2 + 0.3% L-lysine	55.8bc	4.77
4. 11% protein (1004 M.E. Cal./lb.)[3]	21.6a	11.98
5. As 4 + 0.15% DL-methionine	29.6a	9.75
6. As 5 + 0.11% L-lysine	22.5a	9.75
7. As 6 + 0.02% DL-tryptophan	28.8a	9.63
8. As 7 + 0.08% DL-isoleucine	25.4a	10.48
9. As 8 + 0.25% L-arginine	29.8a	9.30
10. As 9 + 0.12% DL-valine	21.8a	11.20
11. As 4 + amino acid mixture[4]	27.9a	10.07
12. 16% protein[5]	63.8c	3.95

[1] Each treatment consisted of approximately 15 hens.

[2] Basal Diet 220, composition given in Table 1, page 23.

[3] Basal Diet 224, composition given in Table 1, page 23.

[4] This amino acid mixture was equivalent to 75% of the level of amino acids added to treatment 10.

[5] Basal Diet 221, composition given in Table 1, page 23.

[6] Values having different subscripts are significantly different at the 0.05 level of probability.

Egg weight with Diet 224, treatment 4, was significantly lower than with the 16 percent protein group, treatment 12. The Haugh Units were higher with the smaller eggs as might be expected since fewer eggs were produced by these groups. Methionine seemed to improve egg weight slightly although not significantly.

Body weight loss was greater with Diet 224 than Diet 220. This might be expected due to the lower energy content of this diet. Body weight changes were not beneficially affected by the additions of amino acids to Diet 224. Losses were greatest with the groups receiving the added lysine and valine.

The results from experiments 2a and 2b indicated that lysine is the first limiting amino acid in Diet 220; whereas methionine is the first limiting amino acid in the lower energy, low protein diets 222 and 224. Since beneficial response was obtained from the addition of methionine to the low energy, low protein diets, a response from the addition of methionine to Diet 221 might also be expected due to the fact that Diets 222, 224 and 221 have a similar Calorie to protein ratio and amino acid pattern. However, this was not demonstrated in experiments 1a and 1b.

Experiment 3a

Experiments 3a and 3b were conducted to determine which amino acids other than methionine and lysine might be limiting for egg production in Basal Diet 220. The amino acid additions were made at levels necessary to bring the total dietary content to a minimum of

Table 15. Weight and Haugh Units of Eggs and Body Weight Maintenance of Hens Fed a High Protein and High and Low Energy, Low Protein Diets Supplemented With Amino Acids.

Experiment 2b

Treatment[1]	Egg Weight	Haugh Units	Body Weight Change	Final Weight
	gms./egg		gms./hen	gms./hen
1. 11% protein (1421 M.E. Cal./lb.)[2]	58ab[6]	80bcd	-133f	1837
2. As 1 + 0.2% DL-methionine	58ab	72a	-201e	1616
3. As 2 + 0.3% L-lysine	57ab	80bcd	-176ef	1701
4. 11% protein (1004 M.E. Cal./lb.)[3]	55a	80bcd	-252cde	1647
5. As 4 + 0.15% DL-methionine	57ab	82d	-270cd	1610
6. As 5 + 0.11% L-lysine	56ab	81cd	-304bc	1476
7. As 6 + 0.02% DL-tryptophan	56ab	77abcd	-315b	1552
8. As 7 + 0.08% DL-isoleucine	54a	83d	-348ab	1519
9. As 8 + 0.25% L-arginine	58ab	80bcd	-231de	1584
10. As 9 + 0.12% L-valine	57ab	82d	-386a	1462
11. As 4 + amino acid mixture[4]	56ab	74ab	-354ab	1514
12. 16% protein[5]	60b	75abc	123g	1908

[1] The data given for egg weight and Haugh Units are the averages of four days production during the sixth month of the experiment.
[2] Basal Diet 220.
[3] Basal Diet 224.
[4] This amino acid mixture was equivalent to 75% of the level of amino acids added to treatment 10.
[5] Basal Diet 221.
[6] Values having different subscripts are significantly different at the 0.05 level of probability.

125 percent of the requirements as suggested by Johnson and Fisher (1958). These suggested requirements are shown in Table 2, page 25. The egg production and feed efficiency data for experiment 3a are presented in Table 16, page 52. A statistically significant response in egg production was obtained with treatment 6. It is not possible to determine from this data whether this response was due to the addition of the 0.05 percent DL-tryptophan _per se_ or to the combination of amino acids which were added to this treatment group. No beneficial effect on egg production or feed efficiency was exhibited with cumulative additions of glycine, valine, arginine or isoleucine. Valine depressed egg production and feed efficiency, though the reduction was not significantly different from the basal group.

Egg weights, Table 17, page 53, were significantly lower with the addition of arginine than with any of the other groups. The tryptophan group produced slightly larger eggs than any of the other treatments. The Haugh Unit measurement of eggs was not significantly affected by the dietary treatments.

Body weight loss during the experimental period was greatest with treatment 2, the group receiving the glycine addition. Isoleucine seemed to beneficially affect body weight maintenance as did tryptophan. The treatment group receiving tryptophan gained body weight during the experiment while all of the other groups lost weight. Again it is not possible to determine from these data whether or not the differences in body weight maintenance in treatments 5 and 6 were due to

Table 16. Hen-day Egg Production and Feed Efficiency of Hens Fed a Low Protein Diet Supplemented With Amino Acids for Six Months.

Experiment 3a

Treatment[1]	Egg Production	Feed Efficiency
	%	lbs./doz.
1. 11% protein[2] + 0.2% DL-methionine and 0.3% L-lysine	45.6a[3]	5.50a
2. As 1 + 0.2% glycine	44.6a	5.22a
3. As 2 + 0.2% DL-valine	35.8a	6.40a
4. As 3 + 0.4% L-arginine	45.5a	5.55a
5. As 4 + 0.4% DL-isoleucine	46.8a	5.56a
6. As 5 + 0.05% DL-tryptophan	59.1b	4.78a

[1] Each treatment consisted of 10 hens, individually fed.
[2] Basal Diet 220, composition given in Table 1, page 23.
[3] Values having different subscripts are significantly different at the 0.05 level of probability.

Table 17. Weight and Haugh Units of Eggs and Body Weight Maintenance of Hens Fed a Low Protein Diet Supplemented With Amino Acids.

Experiment 3a

Treatment[1]	Egg Weight	Haugh Units	Body Weight Change	Final Weight
	gms./egg		gms./hen	gms./hen
1. 11% protein[2] + 0.2% DL-methionine and 0.3% L-lysine	57bc[3]	84a	-185ab	1700
2. As 1 + 0.2% glycine	57bc	82a	-244a	1639
3. As 2 + 0.2% DL-valine	56bc	79a	-159ab	1636
4. As 3 + 0.4% L-arginine	50a	78a	-169ab	1712
5. As 4 + 0.4% DL-isoleucine	54b	77a	-73ab	1727
6. As 5 + 0.05% DL-tryptophan	59c	77a	+80b	1862

1 The data presented for egg weight and Haugh Units are the averages of 2 days egg production during the sixth month of the experiment.

2 Basal Diet 220.

3 Values having different subscripts are significantly different at the 0.05 level of probability.

isoleucine and tryptophan or to the combinations of amino acids added to these treatments.

Experiment 3b

Experiment 3b was carried out in an attempt to determine whether the responses obtained in experiment 3a were due to tryptophan alone or the combinations of amino acids which had been added to Basal Diet 220. In this experiment as shown in Table 18, page 55, tryptophan was added singly and in combination with other amino acids to the low protein, high energy basal diet. An increase in both egg production and feed efficiency was obtained with the tryptophan addition. The differences, however, were not statistically significant. The additions of valine and isoleucine did not affect egg production or feed efficiency. Arginine reduced egg production and feed efficiency. In treatment 6, tryptophan, valine, arginine and isoleucine were added at 75 percent of the level included in treatment 5 with no difference in results. This would indicate that a supplemental level of 0.0375 percent DL-tryptophan to Diet 220 was adequate for egg production under these conditions.

Egg weight, Table 19, page 56, differed only for treatment 4, being significantly lower for this group in comparison to any of the other treatments. Haugh Units were variable being highest for the small egg groups and lowest with the large egg groups. Although there were large differences in body weight maintenance between groups, none of these differences were statistically significant due to the

Table 18. Hen-day Egg Production and Feed Efficiency of Hens Fed a Low Protein Diet Supplemented With Amino Acids for Five Months.

Experiment 3b

Treatment[1]	Egg Production	Feed Efficiency
	%	lbs./doz.
1. 11% protein[2] + 0.2% DL-methionine and 0.3% L-lysine	47.4a[3]	5.66a
2. As 1 + 0.05% DL-tryptophan	56.1a	4.93a
3. As 2 + 0.2% DL-valine	55.5a	4.97a
4. As 3 + 0.4% L-arginine	48.7a	5.42a
5. As 4 + 0.4% DL-isoleucine	58.7a	4.70a
6. As 1 + 0.0375% DL-tryptophan, 0.1500% DL-valine, 0.3000% DL-arginine, 0.3000% DL-isoleucine	58.9a	4.78a

[1] Each treatment consisted of 10 hens, individually fed.
[2] Basal Diet 220, composition given in Table 1, page 23.
[3] Values having different subscripts are significantly different at the 0.05 level of probability.

Table 19. Weight and Haugh Units of Eggs and Body Weight Maintenance of Hens Fed a Low Protein Diet Supplemented With Amino Acids.

Experiment 3b

Treatment[1]	Egg Weight	Haugh Units	Body Weight Change	Final Weight
	gms./egg		gms./hen	gms./hen
1. 11% protein[2] + 0.2% DL-methionine and 0.3% L-lysine	58b[3]	78ab	2a	1701
2. As 1 + 0.05% DL-tryptophan	58b	78ab	217a	1884
3. As 2 + 0.2% DL-valine	59b	78ab	87a	1787
4. As 3 + 0.4% L-arginine	54a	80b	-25a	1668
5. As 4 + 0.4% DL-isoleucine	60b	75a	36a	1761
6. As 1 + 0.0375% DL-tryptophan, 0.1500% DL-valine, 0.3000% DL-arginine, 0.3000% DL-isoleucine	58b	77ab	92a	1753

1 The data for egg weight and Haugh Units are the averages of four days egg production during the fifth month of the experiment.

2 Basal Diet 220

3 Values having different subscripts are significantly different at the 0.05 level of probability.

variation within treatments. Those hens receiving the addition of tryptophan singly, gained the most body weight while the basal group essentially maintained their initial weight throughout the experiment. The toxic effect of arginine was demonstrated on body weight maintenance as well as on egg production, feed efficiency and egg weight.

From experiments 3a and 3b, it might be concluded that, in addition to methionine and lysine, tryptophan is also limiting for egg production in a high energy, 11 percent protein corn-soybean oil meal ration. Although there is little previous research reported in the literature to substantiate this, the work by Waibel and Johnson (1961) did indicate that a slight improvement in egg production was obtained when 0.04% DL-tryptophan was added to a 10 percent protein diet containing added methionine and lysine.

Further research is necessary to determine the optimum supplemental levels of methionine, lysine and tryptophan to low protein diets. Once these levels are determined, a comparison between a properly supplemented, 11 percent protein diet and a 16 percent protein diet should indicate whether or not other amino acids might be limiting for egg production in the low protein ration.

GENERAL DISCUSSION

The lack of any improvement in reproductive performance when methionine or lysine was added to the 16 percent protein diets (221 and 223) suggests that these diets were adequate in these amino acids. Under conditions similar to those in this work where the rate of egg production averages about 60 percent, the diet contains about 1400 Calories of metabolizable energy per pound, and the protein is derived from corn and soybean oil meal, no amino acid supplementation would appear to be warranted.

However, where the 16 percent protein diet was diluted with fiber, as in Diet 222, and consequently where the amino acid balance was similar, a significant response to methionine was obtained. This suggests that the 16 percent protein corn-soybean type diet was somewhat deficient in methionine for maximum efficiency. The lack of response from the addition of methionine might be explained on the basis that the hens on the 16 percent protein apparently consumed more protein than needed and thus were able to meet their methionine requirements. Had the energy content of the 16 percent protein diets been higher and consequently consumption lower, a response from methionine might have been obtained. This has been shown by Heywang and co-workers (1963).

On the other hand, where the protein was decreased by reducing soybean oil meal and increasing corn as in Diet 220 and the amino acid balance altered, deficiencies of lysine, methionine and tryptophan were demonstrated. This shows that corn protein is deficient in these amino acids. As further extension of soybean protein is made by dilution with corn, supplementation with amino acids becomes essential. However, use of low protein (mainly corn protein) diets supplemented with lysine, methionine and tryptophan cannot yet be recommended, since maximum reproductive performance was not obtained.

SUMMARY

Experiments were conducted over a period of two years to study the effects of amino acid supplementation of low and high energy and low and high protein diets on the performance of SCWL laying hens. This research was conducted in two different environmental conditions; one a litter floor, cold-wall poultry house, the other a warm-wall house with individual cages.

Under the former environment, methionine and lysine were added singly and in combination to a 16 percent protein diet containing 1414 Calories of metabolizable energy per pound and methionine singly and a combination of methionine and lysine to a 16 percent protein diet containing 1340 Calories of metabolizable energy per pound. These dietary treatments did not beneficially affect egg production, feed efficiency, body weight, egg weight, Haugh Units, fertility or hatchability of fertile eggs. In one of the experiments, the addition of lysine did improve Haugh Units significantly over the basal control group.

With an 11 percent corn-soybean oil meal type ration containing 0.2 percent added DL-methionine, the addition of 0.3 percent L-lysine improved slightly, though not significantly, egg production, feed efficiency, and egg weight. Haugh Units, fertility and hatchability of fertile eggs were not affected.

Egg production and feed efficiency for the methionine plus

lysine supplemented 11 percent protein diet were inferior to that obtained with the 16 percent protein diets. In addition, the low protein groups lost body weight during the experimental periods while the high protein groups gained weight. Fertility and hatchability of fertile eggs were not affected by protein level.

In the warm-wall, individual cage environment, the effect of amino acid supplementation of low protein diets upon laying performance was studied. No benefit from the addition of methionine singly to a low protein, high energy diet (mostly corn) was observed. However, egg production and feed efficiency were improved with combined additions of methionine and lysine to this diet. Body weight, egg weight and Haugh Units were not affected. Egg production and feed efficiency of the methionine plus lysine supplemented 11 percent diet were inferior to that obtained from hens receiving a 16 percent protein diet containing the same level of energy.

Cumulative additions of methionine, lysine, glycine, valine, arginine, isoleucine, and tryptophan were made to a 10 percent protein, low energy diet containing 1001 Calories of metabolizable energy per pound. Similarly, in another experiment, methionine, lysine, tryptophan, isoleucine, arginine and valine were cumulatively added to an 11 percent protein diet containing 1004 Calories of metabolizable energy per pound. In both of these experiments, methionine improved egg production and feed efficiency. None of the other amino acids affected laying hen performance. The low protein groups lost body weight in both of the experiments. Hens receiving a 16 percent protein diet gained weight in both experiments.

When amino acids were added to an 11 percent protein, high energy diet, containing added methionine and lysine, responses in egg production, feed efficiency and body weight maintenance, were obtained from the addition of 0.05 percent DL-tryptophan. Egg weight and Haugh Units were not significantly affected.

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